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Tamoxifen chemotherapy is widely employed to treat estrogen receptor (ER) positive breast cancers. Although this therapy is initially successful, the majority of tumors become resistant to tamoxifen within a few years. The development of resistance to tamoxifen is thought to require interactions between ERs and coactivator proteins, which link ERs to the general transcriptional machinery. Thus, the discovery of novel drugs that block these protein-protein interactions may provide a new strategy to combat drug-resistance. This proposal is directed at the development of techniques needed to identify novel small molecules that block these critical interactions in vivo. The future discovery of these types of compounds may allow evaluation of this strategy in animal models of drug-resistant breast cancer.

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Introduction

There exists an urgent need for new drugs that halt the progression of tamoxifen-resistant breast cancers. The recent discovery of peptides that block interactions between tamoxifen-bound estrogen receptors (ER) and steroid receptor coactivator (SRC) proteins bearing an MFDFF peptide motif represents an important initial step toward this goal.[1] Since peptides do not possess sufficient metabolic stability and cellular permeability to be investigated in animal models of drug-resistant breast cancers, our laboratory is working to identify metabolically stable and cell permeable *peptoid* (N-alkylglycine) peptidomimetics that block interactions between SRC proteins and tamoxifen-bound ERs. We are working to employ combinatorial chemical methods and rational design to discover compounds that inhibit gene expression activated by tamoxifen-bound ERs in cell culture. These compounds have the potential to allow future evaluation of this strategy in animal models of tamoxifen resistant breast cancer.

Body

MALDI Sequencing of Compound "Hits"

This Concept Award funded the development of methods to sequence compound "hits" using matrix assisted laser desorption ionization (MALDI) mass spectrometry.[2] This approach involves capping a small fraction (~ 5 %) of the growing *peptoid* oligomer at each coupling step to generate a series of truncation products that can be detected by mass spectrometry upon cleavage of a single synthesis bead. As shown in Figure 1, we have developed a new method for on-bead *peptoid* sequencing that involves the use of 1:1 acetic acid:bromoacetic acid during *peptoid* synthesis (unpublished results). Acetic acid was found empirically to be approximately $1/20^{th}$ as reactive as bromoacetic acid during DIC-mediated amide bond formation, which results in capping of ~ 5 % of the amino groups on the resin during each coupling cycle using this 1:1 mixture of

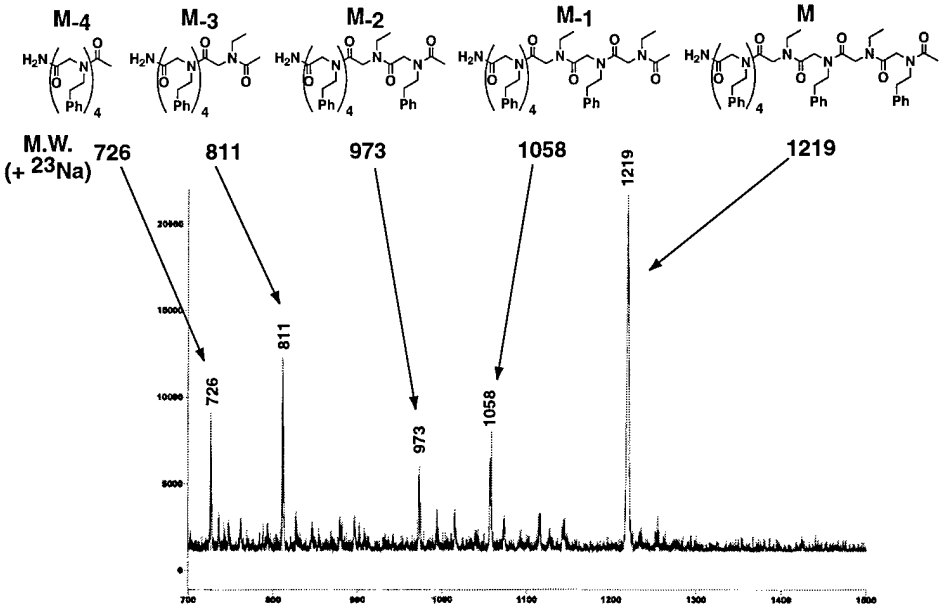

Figure 1. MALDI mass spectral sequencing of a *peptoid* on a single 90 μm tentagel bead. Synthesis of the first four monomer units was followed by addition of acetic acid / bromoacetic acid (1:1) to partially truncate (~ 5 % per cycle analyzed by HPLC) the growing *peptoid* chain. A single bead was spotted on the MALDI plate and compounds released with a stream of TFA vapor. The bead was overlaid with matrix spiked with NaCl for analysis of M+Na peaks. Observed truncated product mass values and differences can unambiguously identify an unknown *peptoid* sequence.

carboxylic acids. After synthesis and on-bead screening, individual beads are selected for analysis and the primary product and minor truncation products cleaved from the resin with trifluoroacetic acid (TFA). Subsequent MALDI mass spectral analysis of these products enables sequencing of the *peptoid* present on a single bead through identification of the primary and truncated product masses.

Development of Whole-Cell Transcriptional Assays

This concept award also funded the development of whole cell estrogen receptor (ER) reporter gene assays using the mammalian cell lines CHO-K1 and SKN-BE2C. As shown in Figure 2, our laboratory developed assays that quantify ER-mediated gene expression at ERE DNA sites in two different mammalian cell lines. In addition to the development of mammalian cell-based assays, we also recently investigated interactions between MFDFF peptides and the

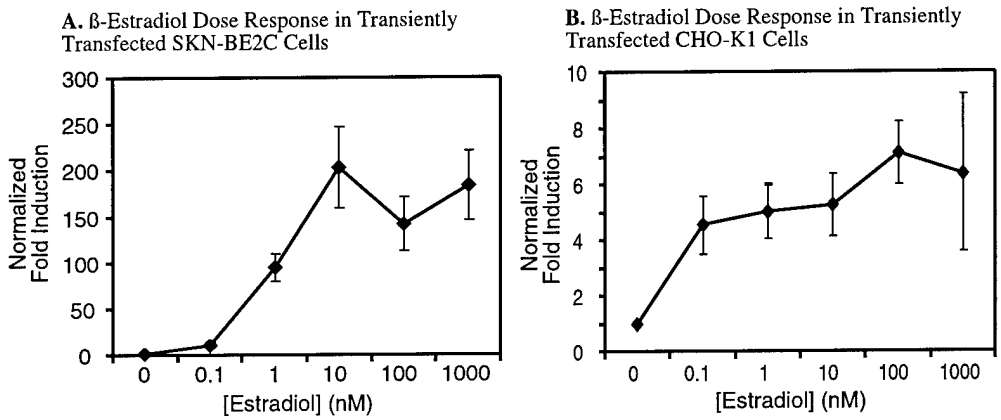

Figure 2. ERα-Mediated transcriptional activity measured in two mammalian cell lines. Both cell lines were cotransfected with pSG5-hERα, pGL2-3ERE-Luc, and pCMV-ßGal. Normalized data are presented in triplicate as fold induction, which represents the ratio of ligand induced activity versus vehicle induced activity for each transfection.

ligand binding domain of ERα using yeast two hybrid assays. As shown in Figure 3, Tamoxifen induces reporter gene expression by 5.5-fold when added to yeast cells coexpressing the ER LBD fused to the bacterial LexA DNA binding domain and the MFDFF peptide fused to the B42 bacterial activation domain. Control experiments revealed that this effect requires the presence of the MFDFF peptide. This yeast-based assay provides an alternative method to confirm whether synthetic compounds identified through library screening competitively inhibit interactions beween ERs and MFDFF peptides.

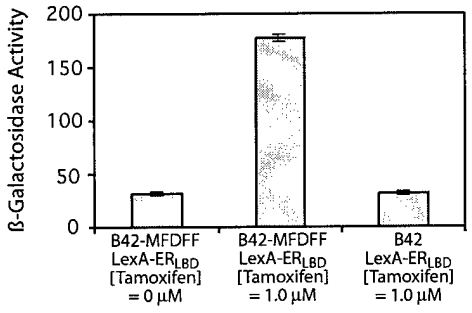

Figure 3. Detection of tamoxifen-dependent MFDFF - ER ligand binding domain (LBD) interactions with a yeast two-hybrid assay. Reporter gene expression from vector pSH18-34 was analyzed in tamoxifen-treated or vehicle-treated *S. Cerevisiae* FY250 coexpressing LexA-ER$_{LBD}$ and B42-MFDFF or B42 alone.

Key Research Accomplishments

Key research accomplishments include (1) the development of a novel method to sequence peptidomimetics on individual synthesis beads by MALDI mass spectrometry, (2) the development of whole-cell reporter gene assays of ligand-induced ER-mediated gene expression, and (3) detection of ER-MFDFF interactions in a yeast two hybrid assay.

Reportable Outcomes

The preliminary data obtained as a result of this Concept Award formed the basis of an American Cancer Society Research Scholar Grant For Beginning Investigators. This grant application, entitled "Synthetic Inhibitors of ER-SRC interactions: Combating Tamoxifen Resistance" (Blake R. Peterson – P.I.) was approved and funded for $427,000 (1/1/02-12/31/04).

Conclusions

This concept award successfully funded the generation of preliminary data that enabled the submission and receipt of a larger and longer-term grant.

References

1. Norris, J. D. et al. Peptide antagonists of the human estrogen receptor. *Science* **285**, 744-746 (1999).

2. Cho, C. Y. et al. Synthesis and screening of linear and cyclic oligocarbamate libraries. Discovery of high affinity ligands for GPIIb/IIIa. *J. Am. Chem. Soc.* **120**, 7706-7718 (1998).

Appendices - None